

WHAT IS CLAIMED IS:

1. A method for treating infections, tumors and autoimmune and inflammatory diseases, comprising administering an effective amount of a polyol-interferon- $\beta$  conjugate having a polyol moiety covalently bound to Cys<sup>17</sup> of human interferon- $\beta$  to a subject in need thereof.

2. The method according to claim 1, wherein said polyol moiety is a polyalkylene glycol moiety.

3. The method according to claim 2, wherein said polyalkylene glycol moiety is a polyethylene glycol (PEG) moiety.

4. The method according to claim 1, wherein the polyol-interferon- $\beta$  conjugate has the same or higher interferon- $\beta$  activity as native human interferon- $\beta$ .

5. A process for producing a polyol-interferon- $\beta$  conjugate having a polyol moiety covalently bound to Cys<sup>17</sup> of human interferon- $\beta$ , comprising:

reacting interferon- $\beta$  with a thiol-reactive polyol agent to site specifically and covalently attach a polyol moiety to Cys<sup>17</sup> of human interferon- $\beta$  to produce a polyol-interferon- $\beta$  conjugate; and

recovering the produced polyol-interferon- $\beta$  conjugate.

6. The process according to claim 5, wherein the thiol-reactive polyol agent is a thiol-reactive PEGylating agent.



7. The process according to either claim 5 or claim 6, wherein the thiol-reactive polyol agent is mono-methoxylated.

8. The process according to either claim 5 or claim 6, wherein the thiol-reactive polyol agent is bifunctional.

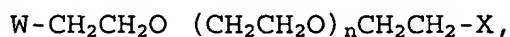
9. The process according to either claim 5 or claim 6, wherein the thiol-reactive polyol agent is a polyol derivative having a functional group selected from the group consisting of orthopyridyl disulfide, vinyl sulfone, maleimide, and iodoacetimide.

10. The process according to either claim 5 or claim 6, wherein the thiol-reactive polyol agent is an orthopyridyl disulfide derivative of a mono-methoxylated polyol.

11. The process according to claim 5, wherein the reacting step is carried out at an acidic pH where interferon-ss is stable.

12. A method for stepwise attachment of polyethylene glycol (PEG) moieties in series to a polypeptide, comprising the steps of:

reacting a polypeptide with a low molecular weight heterobifunctional or homobifunctional PEG moiety having the following formula:

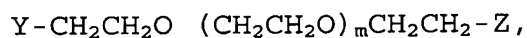




where W and X are groups that independently react with an amine, sulfhydryl, carboxyl or hydroxyl functional group to attach the low molecular weight PEG moiety to the polypeptide; and

reacting the low molecular weight PEG moiety attached to the polypeptide with a monofunctional or bifunctional PEG moiety to attach the monofunctional or bifunctional PEG moiety to a free terminus of the low molecular weight PEG moiety and form a PEG-polypeptide conjugate.

13. The method according to claim 12, wherein the monofunctional or bifunctional PEG moiety has the following formula:



wherein Y is reactive to a terminal group on the free terminus of the low molecular weight PEG moiety attached to the polypeptide and Z is -OCH<sub>3</sub> or a group reactive with X to form a bifunctional conjugate.

14. The method according to claim 13, wherein the monofunctional or bifunctional PEG moiety is methoxy PEG, branched PEG, hydrolytically or enzymatically degradable PEG, pendant PEG, or dendrimer PEG.

15. The method according to claim 12, wherein W and X are independently selected from the group consisting of orthopyridyl disulfide, maleimides, vinylsulfones, iodoacetamides, hydrazides, aldehydes, succinimidyl esters,



epoxides, amines, thiols, carboxyls, active esters, benzotriazole carbonates, p-nitrophenol carbonates, isocyanates, and biotin.

16. The method according to claim 12, wherein the low molecular weight PEG moiety has a molecular weight in a range of about 100 to 5,000 daltons.

17. The method according to claim 12, wherein the monofunctional or bifunctional PEG moiety has a molecular weight in a range of about 100 daltons to 200 kilodaltons.

18. The method according to claim 12, wherein the low molecular weight PEG moiety and/or the monofunctional or bifunctional PEG moiety is a copolymer of polyethylene glycol.

19. The method according to claim 18, wherein the copolymer of polyethylene glycol is selected from the group consisting of polyethylene glycol/polypropylene glycol copolymers and polyethylene glycol/poly (lactic/glycolic acid) copolymers.

20. The method according to claim 12, further comprising a step of purifying the PEG-polypeptide conjugate following the stepwise attachment of two PEG moieties in series to a polypeptide.

21. The method according to claim 20, wherein said step of purifying comprises one or more purification techniques selected from the group consisting of ion exchange chromatography, size exclusion chromatography, hydrophobic



interaction chromatography, affinity chromatography, and reverse phase chromatography.

22. The method according to claim 12, wherein the polypeptide is interferon- $\beta$ .